AD-A144 424 TOPICAL HAZARD EVALUATION PROGRAM OF HEXAHYDRO-1-((2-METHYLCYCLOHEXYL) CA.. (U) ARMY ENVIRONMENTAL HYGIENE AGENCY ABERDEEN PROVING GROUND MD 1/1

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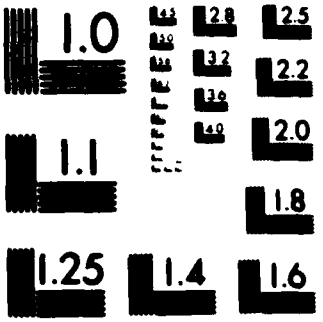

UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010-5422

TOPICAL HAZARD EVALUATION PROGRAM
OF
HEXAHYDRO-1-[(2-METHYLCYCLOHEXYL)
CARBONYL]-1-H-AZEPINE (AI3-35770)
US DEPARTMENT OF AGRICULTURE
CANDIDATE INSECT REPELLENT
STUDY NOS. 75-51-0366-84, 75-51-0435-84
AND 75-51-0489-84
APRIL 1982 - APRIL 1984

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Hexahydro-1-[(2-methylcyclohexyl)carbonyl]-1-H-azepine
USDA Candidate Insect Repellent
Topical Hazard Evaluation Program
AI3-35770
Skin Irritation
Eye Irritation
Photochemical Irritation
Guinea Pig Sensitization

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

All sample batches of hexahydro-1-[(2-methylcyclohexyl) carbonyl]-1-H-azepine, Candidate Insect Repellent AI3-35770, produced moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion. This lesion was characterized by a palpable thickening of the skin, hyperkeratosis, with eventual sloughing of the application site. Ethanol solutions of these chemicals were moderately irritating to the intact skin, and did not produce as severe a response as the technical chemicals. Washing the skin with soap and water, 6 hours after application, of either the technical chemical or ethanol solutions did not

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20. reduce the severity of dermal irritation.

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

CPT(P) Wade/cvc, AUTOVON
584 3980

REPLY TO
ATTENTION OF

HSHB-LT/WP

9 AUG 1984

SUBJECT: Topical Hazard Evaluation Program of Hexahydro-1-[(2-methylcyclohexyl) carbonyl]-1-H-azepine (AI3-35770), US Department of Agriculture, Candidate Insect Repellent, Study Nos. 75-51-0366-84, 75-51-0435-84, and 75-51-0489-84, April 1982 - April 1984

Executive Secretary
Armed Forces Pest Management Board
Forest Glen Section, WRAMC
Washington, DC 20307

EXECUTIVE SUMMARY

The purpose, essential findings, and major recommendations of the inclosed report follow:

a. Purpose. The purpose of this program is to provide guidance for further entomological testing of hexahydro-1-[(2-methylcyclohexyl) carbonyl]-1-H-azepine, (AI3-35770), US Department of Agriculture, candidate insect repellent.

b. Essential Findings. All sample batches of hexahydro-1-[(2-methyl-cyclohexyl)-carbonyl]-1-H-azepine, candidate insect repellent AI3-35770, produced moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion. This lesion was characterized by a palpable thickening of the skin, hyperkeratosis, with eventual sloughing of the application site. Ethanol solutions of these chemicals were moderately irritating to the intact skin, and did not produce as severe a response as the technical chemicals. Washing the skin with soap and water, 6 hours after application, of either the technical chemical or ethanol solutions did not reduce the severity of dermal irritation.

c. Recommendation. Recommend that chemical AI3-35770 be disapproved for further testing as a candidate insect repellent.

FOR THE COMMANDER:

for Rodney M. Attack

JOEL C. GAYDOS, M.D.
Colonel, MC
Director, Occupational and Environmental Health

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as

CF:
HQDA (DASG-PSP) wo incl
Cdr, HSC (HSCL-P)
Comdt, AMS (HSHA-IPM)
Dir, Advisory Cen on Tox, NCR (2 cy)
USDA, ARS, Southern Region (3 cy)
USDA, ARS (Dr. Terrence McGovern)
Cdr, USAMRDC [SGRD-OPM/LTC(P) Reinert]

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

REPLY TO ATTENTION OF

HSHB-LT/WP

TOPICAL HAZARD EVALUATION PROGRAM OF HEXAHYDRO-1-[(2-METHYLCYCLOHEXYL) CARBONYL]-1-H-AZEPINE (AI3-35770) US DEPARTMENT OF AGRICULTURE CANDIDATE INSECT REPELLENT STUDY NOS. 75-51-0366-84, 75-51-0435-84 AND 75-51-0489-84*† APRIL 1982 - APRIL 1984

1. AUTHORITY.

a. Letter, US Department of Agriculture - Agricultural Research Service, Northeastern Region, Beltsville Agricultural Research Center, Beltsville, Maryland, 9 April 1982.

b. Letter, US Department of Agriculture - Agricultural Research Service, Northeastern Region, Beltsville Agricultural Research Center, Beltsville, Maryland, 9 May 1983.

c. Letter, US Department of Agriculture - Agricultural Research Service, Northeastern Region, Beltsville Agricultural Research Center, Beltsville, Maryland, 12 January 1984.

d. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the US Department of Agriculture, Agricultural Research, Science and Education Administrations; titled Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

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* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals;" US Department of Health, Education, and Welfare; Public Health Service; National Institutes of Health (NIH) Publication No. 80-23, revised 1978, reprinted April 1980.

† The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

2. REFERENCE. Toxicology Division Topical Hazard Evaluation Program Procedural Guide, US Army Environmental Hygiene Agency (USAEHA), January 1982.

3. PURPOSE. The purpose of this program is to provide guidance for further entomological testing of hexahydro-1-[(2-methylcyclohexyl) carbonyl]-1-H-azepine, (AI3-35770), US Department of Agriculture (USDA), candidate insect repellent.

4. GENERAL.

a. Initial Primary Irritation Evaluation Program (PIEP) testing was conducted by this Agency in 1973 (AI3-35770-aGa, Study No. 51-018-74, 28 November 1973). Recommendation was made that this chemical be given further consideration as a candidate insect repellent based on the results of primary dermal irritation studies (USAEHA Category II, ref. Appendix A).

b. Further entomological testing conducted by the USDA - Agricultural Research, Southern Region, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida, indicated that this chemical might be promising as an insect repellent. Additional quantities were submitted in April 1982 for Topical Hazard Evaluation Program testing (AI3-37550-e). Due to the time interval, dermal irritation studies were repeated on this batch of chemical, with markedly different results than obtained in initial testing.

c. Due to the USDA priority status of chemical AI3-35770 numerous attempts were made by Dr. Terrence P. McGovern, Organic Chemical Synthesis Laboratory, USDA-Agricultural Research Service, Northeastern Region, Beltsville Agricultural Research Center, Beltsville, Maryland, to determine a possible sample contaminant. This effort resulted in submission of seven additional samples differing in either method of synthesis and/or purification. This report will present an evaluation of the toxicity data developed during studies performed with various batches of chemical AI3-35770.

5. MATERIALS AND METHODS.

a. Hazard evaluations of the USDA candidate insect repellent AI3-35770, were conducted by this Agency using New Zealand White rabbits and albino Hartley guinea pigs. Rabbits and guinea pigs were purchased from Hazleton-Dutchland Laboratories, Denver, Pennsylvania.

b. The samples tested were designated as follows:

Sample	Study Number
AI3-35770-e	75-51-0366-84
AI3-35770-f	75-51-0435-84
AI3-35770-1	75-51-0435-84
AI3-35770-2	75-51-0435-84
AI3-35770-3	75-51-0435-84
AI3-35770-4	75-51-0435-84
AI3-35770X	75-51-0435-84
AI3-35770-g	75-51-0435-84

c. Samples were synthesized and submitted by Dr. Terrence P. McGovern, Organic Chemical Synthesis Laboratory with the exception of AI3-35770X, which was submitted in confidence by a commercial chemical manufacturer upon request of the Armed Forces Pest Management Board. Letters and numbers represent different synthesized and/or purified batches of chemical.

d. The denatured ethanol used in the studies described was manufactured in accordance with Interim Federal Specification O-E-11760C, Ethyl Alcohol (Ethanol); Denatured alcohol; Proprietary Solvents and Special Industrial Solvents.

6. RESULTS.

a. Primary Dermal Irritation Studies.

(1) The potential for primary skin irritation was determined by 24-hour application of 0.5 mL technical grade chemical under an occlusive wrap to the intact and abraded skin of six rabbits. Application sites were examined at 24 and 72 hours and at 7 days for evidence of irritation. Application sites were then observed daily until irritation was resolved. Comparison of the responses using Draize's scale for skin reactions is shown in Table B-1, Appendix B. This scale is based on grades of 0 to 4 for erythema and edema formation with a maximum score of 8. A comparison of the skin irritation scores at 24 and 72 hours, the irritation index, and the USAEHA Category is shown in Table B-2, Appendix B. All batches tested produced an initial moderate primary irritation as evidenced by erythema and edema formation. This response was followed by a thickening of skin at the site of application, resulting in formation of a firm, leathery patch between 72 hours and 7 days. These firm patches of skin sloughed at 12 to 15 days postapplication revealing a raw, moist area devoid of hair growth. Full-thickness skin sections were taken at 7 days postapplication of all sites for samples AI3-35770-1,-2,-3 and -4. These tissues were fixed, sectioned, and stained with hematoxylin-eosin. Upon histological evaluation, the pathology noted was characterized as a proliferative dermatitis. The epidermis was thickened by hyperplasia and covered by a crust of parakeratosis and hyperkeratosis which was infiltrated by heterophils. The papillary dermis had a mild infiltrate of variable numbers of macrophages and lymphocytes. Two of the lesions were also ulcerated. There was some variation in the severity of the lesions noted which correlated well with gross observations.

(2) A 25 percent (w/v) solution of samples AI3-35770-f in 95 percent denatured ethanol was applied at a dose of 0.5 mL (125 mg chemical) to the back of each of six rabbits. Application sites were not covered, rabbits were kept in restrainers for 24 hours, and examined for evidence of irritation at 24 and 72 hours and at 7 days. Irritation was graded using Draize's scale as previously noted. Sample AI3-35770-f in ethanol was initially more irritating to intact skin than was the technical compound, however, the irritation scores decreased after 24 hours as this irritation resolved. At 7 days postapplication the skin areas were dry and scaly, but showed no other evidence of irritation. A summary of the skin irritation scores, the irritation index, and the USAEHA Category is shown in Table B-3, Appendix B.

(3) In an effort to determine whether the severe injury noted in primary dermal irritation studies was due to an initial dermatotoxic insult, or continued presence of the chemical on the skin beyond 24 hours postapplication, a modification of the standard primary irritation test was performed using samples AI3-35770-g and AI3-35770X. A 0.5 mL dose of technical chemical and of a 25 percent (w/v) solution in 95 percent denatured ethanol was applied to the intact skin of each of three rabbits. Application sites were not occluded and animals were maintained in restrainers. Six hours postapplication the rabbits were rinsed with warm water and application sites washed twice, gently, using a gauze sponge and Liquid Ivory Soap®. Rabbits were rinsed thoroughly, towel dried, and returned to their cages. Application sites were examined for evidence of irritation at 24 and 72 hours and at 7 days. Irritation was graded using Draize's scale. There was no significant difference in either the type or severity of irritation noted with this procedure and the routine dermal irritation studies. A comparison of skin irritation scores at 24 and 72 hours, irritation index, and USAEHA Category is shown in Table B-4, Appendix B. These results would indicate that either routine washing with soap and water is ineffective in removing this compound from the skin, or that injury produced within the first 6 hours of application is sufficient to produce the severe irritation demonstrated.

b. Photochemical Skin Irritation Studies. Studies were performed to determine if sample AI3-35770-e would produce photochemical irritation. A single 0.05 mL dose of a 25 percent (w/v) solution of the chemical and of a 10 percent (w/v) Oil of Bergamot solution (positive control) in 95 percent ethanol was applied to the intact skin of six rabbits. Five minutes after application, the rabbits were exposed to ultraviolet (UV) light (365 nm) for 30 minutes at a distance of 10-15 cm. Following UV exposure, 0.05 mL of the test solution, positive control, and diluent were applied to additional skin areas to serve as unirradiated control sites. Application areas were examined for evidence of irritation at 24, 48, and 72 hours. Sample AI3-35770-e did not produce photochemical irritation under test conditions. The ethanol solution did, however, produce moderate primary irritation. Positive control application and irradiation produced greater irritant effects than in unirradiated skin areas.

c. Eye Irritation Studies. A study to determine the irritative potential of sample AI3-35770-e to the eyes of rabbits was conducted concomitant with skin irritation studies. A single dose of 0.1 mL technical chemical was placed in the everted lower lid of one eye in each of nine rabbits. Three of the nine rabbits had the eye flushed with warm water for 1 minute, 25 seconds after application. The eyes were examined for occular injury at 24, 48, and 72 hours after treatment. Sample AI3-35770-e produced moderate injury to the cornea and, in addition, some injury to the conjunctiva (USAEHA Category C, ref. Appendix A). Washing with water reduced the severity of injury noted.

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d. Guinea Pig Sensitization. The inherent sensitization potential of samples AI3-35770-e and AI3-35770-f was studied using male albino Hartley guinea pigs. Intradermal (ID) injections of a minimally irritating concentration of each chemical in a mixture containing 1 volume of propylene glycol and 29 volumes of saline were given to 10 test guinea pigs each. A concurrent group of guinea pigs was similarly tested using the known sensitizer, dinitrochlorobenzene (DNCB). Each animal received 10 sensitizing doses over a 3-week period. After a 2-week rest, they were challenged with ID injections of the test chemical or DNCB, as appropriate. Samples AI3-35770-e and AI3-35770-f did not produce a sensitization reaction under test conditions. Challenge doses of DNCB in positive control guinea pigs produced a marked sensitization reaction in 10 out of 10 guinea pigs.

7. DISCUSSION. All sample batches of hexahydro-1-[(2-methylcyclohexyl)-carbonyl]-1-H-azepine, candidate insect repellent AI3-35770, produced moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion. This lesion was characterized by a palpable thickening of the skin, hyperkeratosis, with eventual sloughing of the application site. Ethanol solutions of these chemicals were moderately irritating to the intact skin, and did not produce as severe a response as the technical chemicals. This irritation was noted upon application of as little as 12.5 mg of sample AI3-35770-e (0.05 mL of a 25 percent solution). Washing with soap and water of either the technical chemical or ethanol solutions 6 hours after application did not reduce the severity of dermal irritation. These studies were monitored by the Analytical Quality Assurance Office (see Appendix C).

8. RECOMMENDATION. Recommend that chemical AI3-35770 be disapproved for further testing as a candidate insect repellent (paragraph 1d, this report).

JOHN V. WADE, DVM
CPT(P), VC
Laboratory Animal Veterinary Officer
Toxicology Division

JOHN G. HARVEY, JR
Biological Laboratory Technician
Toxicology Division

APPROVED:

MAURICE H. WEEKS
Chief, Toxicology Division

APPENDIX A

TOPICAL HAZARD EVALUATION PROGRAM
DEFINITIONS OF CATEGORIES OF COMPOUNDS BEING
CONSIDERED FOR ACUTE SKIN APPLICATION

CATEGORY I - Compounds producing no primary irritation of the intact skin or no greater than mild primary irritation of the skin surrounding an abrasion. (INTERPRETATION: No restriction for acute application to the human skin.)

CATEGORY II - Compounds producing mild primary irritation of the intact skin and the skin surrounding an abrasion. (INTERPRETATION: Should be used only on human skin found by examination to have no abrasions or may be used as a clothing impregnant.)

CATEGORY III - Compounds producing moderate primary irritation of the intact skin and the skin surrounding an abrasion. (INTERPRETATION: Should not be used directly on the skin without a prophetic patch test having been conducted on humans to determine irritation potential to human skin. May be used without patch testing, with extreme caution, as clothing impregnants. Compound should be resubmitted in the form and at the intended use concentration so that its irritation potential can be reexamined using other test techniques on animals.)

CATEGORY IV - Compounds producing moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion and, in addition, producing necrosis, vesiculation, and/or eschars. (INTERPRETATION: Should be resubmitted for testing in the form and at the intended use concentration. Upon resubmission, its irritation potential will be reexamined using other test techniques on animals, prior to possible prophetic patch testing in humans, at concentrations which have been shown not to produce primary irritation in animals.)

CATEGORY V - Compounds impossible to classify because of staining of the skin or other masking effects owing to physical properties of the compound. (INTERPRETATION: Not suitable for use on humans.)

EYE CATEGORIES:

A. Compounds noninjurious to the eye. INTERPRETATION: Irritation of human eyes is not expected if the compound should accidentally get into the eyes, provided it is washed out as soon as possible.

B. Compounds producing mild injury to the cornea. INTERPRETATION: Should be used with caution around the eyes.

C. Compounds producing mild injury to the cornea, and in addition some injury to the conjunctiva. INTERPRETATION: Should be used with caution around the eyes and mucosa.

D. Compounds producing moderate injury to the cornea. INTERPRETATION: Should be used with extreme caution around the eyes.

E. Compounds producing moderate injury to the cornea, and in addition producing some injury to the conjunctiva. INTERPRETATION: Should be used with extreme caution around the eyes and mucosa.

F. Compounds producing severe injury to the cornea and to the conjunctiva. INTERPRETATION: Should be used with extreme caution. It is recommended that use be restricted to areas other than the face.

APPENDIX B

TABLES

TABLE B-1. DRAIZE'S SCALE FOR SCORING SKIN REACTIONS

	Grade
1. ERYTHEMA AND ESCHAR FORMATION	
a. No erythema	0
b. Very slight erythema (barely perceptible)	1
c. Well defined erythema	2
d. Moderate-to-severe erythema	3
e. Severe erythema ("beet" redness to slight eschar formation injurious in depth)	4
f. Possible total erythema score	4*
2. EDEMA FORMATION	
a. No edema	0
b. Very slight edema (barely perceptible)	1
c. Slight edema (edges of areas well defined by definite raising)	2
d. Moderate edema (edges raised approximately 1 mm)	3
e. Severe edema (raised more than 1 mm and extending beyond area of application)	4
f. Possible total edema score	4*
3. POSSIBLE TOTAL SCORE FOR PRIMARY IRRITATION	8

* Any skin reaction more serious than severe edema, vesiculation, ulceration, or necrosis places the chemical in category IV.

TABLE B-2. COMPARISON OF SKIN IRRITATION SCORES FOLLOWING SINGLE APPLICATION TO RABBITS AT 24 AND 72 HOURS

Sample	Mean Irritation 24-Hours	Mean Irritation 72-Hours	Irritation Index	USAEHA Category (ref Appendix A)
AI3-35770-e	3.25	4.67	4.33	IV
AI3-35770-f	1.67	4.33	3.27	IV
AI3-35770-1	2.67	4.33	3.50	IV
AI3-35770-2	2.83	4.50	4.00	IV
AI3-35770-3	2.67	4.33	3.50	IV
AI3-35770-4	3.33	4.67	4.00	IV
AI3-35770X	4.50	7.50	6.00	IV
AI3-35770-g	4.50	7.67	6.10	IV

TABLE B-3. SKIN IRRITATION SCORE FOLLOWING SINGLE APPLICATION OF A 25 PERCENT (W/V) ETHANOL SOLUTION TO RABBITS AT 24 and 72 HOURS

Sample	Mean Irritation 24-Hours	72-Hours	Irritation Index	USAEHA Category (ref Appendix A)
AI3-35770-f	5.33	3.33	4.33	III

TABLE B-4. COMPARISON OF SKIN IRRITATION FOLLOWING SINGLE APPLICATION OF TECHNICAL GRADE CHEMICAL AND ETHANOL SOLUTION, WASHED AT 6-HOURS POSTAPPLICATION

Sample	Mean Irritation 24-Hours	72-Hours	Irritation Index	USAEHA Category (ref Appendix A)
AI3-35770-g (technical)	4.67	7.33	6.00	IV
AI3-35770-g (ethanol)	4.00	5.67	4.83	III
AI3-35770X (technical)	5.00	6.33	5.67	IV
AI3-35770X (ethanol)	3.67	4.67	4.17	III

APPENDIX C

ANALYTICAL QUALITY ASSURANCE

The Analytical Quality Assurance Office certifies the following:

a. These studies were conducted in accordance with:

(1) Standing Operating Procedures developed by the Toxicology Division, USAEHA.

(2) Title 21, Code of Federal Regulations (CFR), 1983 rev, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

(3) Final Rule, Pesticide Programs; Good Laboratory Practice Standards; 48 Federal Register (FR) 53946-53969, 29 November 1983.

(4) Final Rule, Toxic Substances Control; Good Laboratory Practice Standards; 48 Federal Register (FR) 53922-53944, 29 November 1983.

b. Facilities were inspected during its operational phase to ensure compliance with paragraph a above.

c. The information presented in this report accurately reflects the raw data generated during the course of conducting these studies.

Richard W Reynolds
for PAUL V. SNEERINGER, Ph.D.
Chief, Analytical Quality
Assurance Office

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